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IMPACT OF ACUTE TOTAL-BODY GAMMA-IRRADIATION ON SPERM PRODUCTION OF LABORATORY RATS

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Introduction. In the era of assisted reproductive technologies, which intervene natural selection, when there is a risk of fertilization of egg by defective father's genetic material, exists an acute need of deep study of parameters of spermiogram as a main indicator of fertility disorders.

Infertility is an international problem and involves 15% of family couples having unprotected sexual intercourse. Unfortunately, there is no sufficient accurate information indicating the rate of male factor in the whole world. In general, approximately one third of cases are associated with male reproductive failure. Another third includes female reproductive problems, and the last one – both and other unknown factors of infertility [0].

Nowadays, possibility of acute ionizing irradiation (IR) declined according to development of a number of events aimed to prevent and protect in industry, and also by acquired skills of work with sources of radiation. However, increasing production of fissile materials, testing of different atomic devices, nuclear accidents, lead to a global pollution of environment by radioactive substances, to action of which is exposed all population of world.

Roentgen and gamma rays produce a fatal effect on germinal and Leydig's cells in the human body. This kind of radiation can result in irreversible damage leading to infertility [0].

After two, the biggest in the world, nuclear disasters in Chornobyl (1986) and Fukushima (2011) for the last few years many researches began to study effect of lowdose IR on sperm parameters. Unfortunately, till this time, data are controversy and need deep study of this problem.

Meantime, it was shown that constant effect of low-dose around 30 km area of Chornobyl adversely impacts on generative function of outbred rats and at the same time, on postnatal development of last one during several generations. It is indicative by a meaningful reduction of birth and increasing of mortality of immature rats that could be related with elevated anatomical pathology and chromosomal aberrations in cells of these generations [0]. Contradictory data was revealed in a research of influence of environmental radiation on testes and spermatogenesis in wild large Japanese field mice from Fukushima. It was shown, that there was no harmful impact on germ cells of studied animals. As a conclu-

sion, authors suggested that high levels of radioactive contamination can have surprisingly limited effects on spermatogenesis in some species [0].

Spermatozoa develop in testicles as a result of spermiogenesis. Total duration of human spermiogenesis dues 73-77 days, at the same time, this process in rats takes 47-49 days. It is exactly known duration of separate stages of spermatogenesis. For example, in rats, phase of spermatogonia dues 9 days, period of spermatocytes-18-19 days, spermatids-20-21 days, and the last one- phase of mature spermatozoa up to 10 days. Besides, at any period in testicles are present germ cells of all phases of development, that in a case of exposure to radiation leads to injury to all of them [0].

By analysing literature as to the hereditary influence of ionizing radiation on male population indicates that, along with quantity and dose of exposure, principal meaning has a phase of spermatogenesis at the moment of irradiation, and this aspect is of the nature of the general biological pattern [0].

Aim of this study. Considering some contradictory data in the literature concerning impact of IR on reproductive system, our aim was to study and to analyse impact of total single exposure to radiation (dose dependent) on parameters of sperm production of rats.

Object and methods. The experiments were conducted on mature albino laboratory rats 2,5 month of age. The animals were kept under artificial daylight hours (12-hour day/12-hour night) and usual dietary intake (food and water *ad libitum*). The experiments were performed according to the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes.

Total single exposure to radiation of the animals was conducted by means of "ROKUS" apparatus (gammaquantum source – 60 Co; specific absorption rate 106,6 cGy/min) in the doses 0,6; 1,0; 2,0; 3,0 and 5,0 Gy. The animals were decapitated 5, 15, 30 and 65 days after total irradiation. To avoid any irradiation of animals from the control group a special protective lead screen was used (the control group of animals with imitation – control group A) which contained 6 animals and a control group without imitation (control group B) with the same quantity of animals.

Evaluation of Spermatogenesis. After irradiation at certain post-radiation terms 6 animals from every group and 6 animals from the control one were weighed and decapitated by means of a guillotine under mild narcosis. The testicles and epididymis were removed from the animals and used in the further experiments.

The amount of spermatozoa in 1 ml of sample and in the whole sample was calculated in Goryaev chamber under the microscope.

Spermatozoa suspension was preliminary prepared for light microscopy. For this purpose, spermatozoa

Table 1 – Daily production of cells (spermatozoa) x10⁶ by testicles

Day Dose, Gy	5	15	30	65
Control group A	27.21±1.09	31.38±1.14	35.00±0.86	39.57±1.14
Control group B	26.91±0.62	32.65±0.77	35.38±0.76	40.63±1.28
0.6	26.37±1.12	30.11±1.45	32.48±1.15*	41.03±1.50*
1.0	26.78±0.50*	29.55±0.68*	29.55±0.68*	41.91±0.59*
2.0	22.71±0.58*	24.15±1.06*	25.20±0.56*	33.63±0.56*
3.0	17.7±0.58*	20.78±0.33*	17.65±1.00*	13.67±0.83*
5.0	14.76±0.45*	16.01±0.49*	0.096±0.006*	0.027±0.003*

Notes: * – statistically reliable differences with the control $p \le 0.05$.

were squeezed out from the cut epididymis by means of a little stick into NaCl solution. The suspension obtained was filtrated from the epididymis through a metal filter screen and mixed carefully. The suspension was placed into the thermostat at the temperature of 37 °C. Several drops of the suspension were taken with a dropper for the microscopic analysis, and then they were mixed with an equivalent volume of 0,2% formic acid solution. A spermatozoid smear was prepared after preservation. Freshly prepared mixture was applied onto the dried smear. The mixture contained 7 portions of 50 % silver nitrate water solution and 1 portion of 0,2% formic acid.

Then the sample covered with the glass slide was placed in the thermostat in Petri dishes at the temperature of 55-60 °C. Duration of staining was 15-20 minutes. After that the samples were washed with distilled water and dried in the air.

Complimentary samples of spermatozoa were prepared by means of applying a drop of the suspension on the microscopic slide. Water solution containing eosin (0,25%), nigrosine (10%) and sodium chloride (0,9%) was added to the suspension. Then the sam- Notes: * – statistically reliable differences with the control $p \le 0.05$. ple was covered with the glass slide, and vi-

tality of spermatozoa was determined under the light microscope with magnification ×400.

The amount of sperm cell in the testicles was determined by a certain method [0,0]. In accordance with the protocol, decapsulated testicles first were grinded and then homogenized for 2 minutes at a maximum speed of laboratory blenders in the mixture consisting of 150 mM NaCl, 3,8 mM NaN₃ and 0,05% Triton X-100 (v/v). Testicular homogenate was kept for 24 hours at the temperature of 5°C. During this period the heads of spermatids remained in the solution were calculated. Only spermatids of 17-9 stages of spermiogenesis found during IV-VIII stages of the spermatogenetic epithelium cycle are proved to be able to resist not only a destructive power of turbulent flows, occurring with a quick rotation of blenders in water medium, but membranous solubilization by Triton X-100 as well.

General amount of spermatozoa in the epididymis was determined by means of Goryaev chamber and phase-contrast microscopy after homogenization of the epididymis in the saline together with NaN, and Triton X-100. Daily production of spermatozoa by the testicle was calculated by means of division of general amount of turbulent resistant sperm cells in the testicle by duration of their staying. In rats it is 6,1 days [0]. Analysis of kinetic characteristics of spermatozoa was made according to a certain method [0]. According to the protocol, caudal spermatozoa were squeezed out from the second epididymis under mild pressure into the nutrient water solution at the temperature of 37 °C, containing XEMA solution (Sigma) and sodium bicarbonate in the amount of 9,8 g/L and 1,2 g/L, respectively.

Statistical analysis. Results were represented as arithmetic mean ± SEM. The statistical significance of differences between two groups was determined using the t-test with P < 0.05 being considered significant.

Results. The experiments determined the lack of any visible differences in the control groups of animals both with imitation and without imitation of total gamma-irradiation in all the experiments conducted. Therefore, to compare all the data obtained to confirm statistical validity the indices of the control group A were used.

The **table 1** demonstrates that irradiation of animals with the dose of 0.6 Gy provokes inconsiderable decline of a daily production of spermatozoa by the testes contrasting with the control group which is found only on the 30th day after irradiation. And on the 65th day after irradiation the daily production grows to normal indices.

Table 2 – Total amount of cells (spermatozoa) x10⁶ in testicle

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Day Dose, Gy	5	15	30	65
Control group A	181.67±24.01	207.33±15.37	214.5±14.68	226.83±15.50
Control group B	183.83±19.72	207.33±12.61	214.5±13.62	226.0±12.77
0.6	173.5±18.36	202.67±13.81	209.17±16.47	232.5±17.49*
1.0	173.83±17.66	186.17±13.93*	201.0±11.95	252.0±22.23*
2.0	160.83±8.93*	136.67±11.33*	149.33±14.26*	175.0±14.86*
3.0	136.0±11.03*	124.5±5.17*	107.33±5.65*	74.67±9.24*
5.0	78.33±10.46*	30.33±8.52*	0.967±0.118*	0.036±0.009*

A conclusion can be drawn that an elevated dose of irradiation causes a reduced ability of a daily production by the testicles. Thus, with the dose of irradiation 3.0 Gy the daily production on the 5th day becomes 65% only compared to the control group. And on the 65th day with the same dose of irradiation it is 34,5% only.

With the dose 5.0 Gy a sharp growth of a daily sperm production by the testicles in the period from the 5th to the 65th day is observed: from 14.76±0.45 to 0.027±0.003 x106 cells (spermatozoa) respectively. In comparison, on the 65th day a percentage ratio to that of the control is about 0,07%.

The total amount of spermatozoa in the testicles (table 2) with the dose of ionizing radiation 0.6 Gy did not vary reliably from that of the control groups. And on the contrary, on the 65th day an inconsiderable increase of the total amount if observed, which is explained by heightened daily testicular production of spermatozoa during this term (table 1). Similar picture is observed with the dose of irradiation of rats equal to 1.0 Gy. Evaluating of the both daily and total amount of spermatozoa in the testicles of animals killed on the 65th day after irradiation was found. The tables 1 and 2 demonstrate that on the 5th day after irradiation in the dose of 2.0 Gy both indices decrease in comparison with that of the control. And till the 65th day the indices gradually restore, though they still remain lower than that of

the control. General irradiation of rats with the doses 3.0 and 5.0 Gy is reflected during all the terms in a considerable reduction of the total amount of spermatozoa in the testicles

Appropriately, with the dose of irradiation 5.0 Gy (table 2) general decrease of the indices occurs from 78.33±10.46 to 0.036±0.009 x10⁶ cells (spermatozoa), which constitutes 43% and 0.015% from that of the control.

A normal amount of viable spermatozoa in the epididymis (table 3) was found with the dose irradiation 0.6-1.0 Gy during the first five days of the experiment in comparison with the control group containing non-irradiated rats. The amount of viable spermatozoa in the epididymis of the irradiated animals with the similar doses on the 65th day was higher than that in the control. which is explained by the fact that their general amount in the epididymis increases the control considerably (table 4). With the dose of radiation 2.0-5.0 Gy a considerable harmful effect of ionizing radiation was Notes: * - statistically reliable differences with the control p ≤ 0,05. found, which was the most significant with

lethal doses in case of 5.0 Gy. Thus, in case of irradiation doses 0.6-1.0 Gy till the end of the experiment the indices of spermatozoa vital activity returned to the normal values at the expense of their increased total amount in the epididymis (table 3).

General amount of spermatozoa in the epididymis with the doses 0,6 and 1.0 Gy during the first 5 days remained on the control level. On the 5th day in both experiments the indices 3 and 8% decreased in comparison with the control. On the 30th day their general amount became equal to that of the control gradually. At the same time, with the dose of irradiation 1.0 Gy on the 65th day the indices were approximately 13% higher than that of the control (table 4).

On the contrary, with the dose 2.0-5.0 Gy general amount of spermatozoa in the epididymis of rats was considerably lower than that of the control values in all the terms of observation.

With an increased dose of irradiation, the amount of spermatozoa and, as well as, their vital activity decrease considerably (fig. 1). Thus, in the control group the amount of viable spermatozoa in the epididymis is 87% out of their total amount. With the dose 2.0 Gy their number decreases to 15%. The dose of irradiation 3.0 Gy significantly reduced a total number of spermatozoa and the part of viable spermatozoa was 23%. At the same time, approximately 6,3% of viable spermatozoa was found out of total amount of them in the experimental animals received the dose of irradiation 5.0 Gy.

The comparative diagram (fig. 2) on the 65th day, when animals were killed, reflects that both the epididymis and testicles demonstrate similar dose sensitivity to irradiation during general irradiation of animals. With doses 0,6-1.0 Gy an inconsiderable evaluation of the total amount of spermatozoa is detected. With the heightening of dose to 5.0 Gy both indices drop to 0.015% compared to the control groups.

Discussion. Reproductive gonads are among the most sensitive tissues to irradiation. Influence on the

Table 3 – Amount of viable spermatozoa (cells) x106 in epididymis

Day Dose, Gv	5	15	30	65
Control group A	214.30±21.90	236.17±19.55	245.7±17.20	247.00±12.44
Control group B	212.17±10.63	236.17±13.21	246.33±11.40	267.83±17.61
0.6	190.83±17.75	222.17±14.96*	231.67±12.42*	254.00±21.54
1.0	190.00±12.25	206.33±14.46*	222.33±8.43*	282.17±17.81*
2.0	155.00±9.33*	150.83±10.50*	98.67±9.09*	48.83±4.07*
3.0	132.83±6.55*	124.67±5.50*	94.33±4.54*	28.33±3.98*
5.0	32.17±3.19*	12.17±3.19*	0.037±0.006*	0.002±0.0004*

Notes: * – statistically reliable differences with the control $p \le 0.05$.

Table 4 – General amount of cells (spermatozoa) x10⁶ in epididymis

Day Dose, Gy	5	15	30	65
Control group A	249.7±26.5	272.67±17.65	277.67±17.28	283.00±18.70
Control group B	246.17±16.03	273.17±17.46	281.50±11.10	296.67±13.75
0.6	237.67±20.45	264.50±18.88*	272.33±12.25	299.33±14.10
1.0	236.67±11.64	251.00±15.74*	266.67±10.05	320.83±19.82*
2.0	211.83±18.84*	195.33±11.13*	202.50±13.14*	229.00±18.55*
3.0	214.33±34.75*	170.83±8.04*	154.33±8.45*	123.17±6.30*
5.0	125.67±5.61*	79.50±10.67*	1.327±0.09*	0.038±0.003*

gonads is dose- and time- dependent [0] that coincides with our data. Sensitivity of germinal epithelium to damages caused by IR, that was described in literature, reveals that changes to spermatogonia can be detected following doses as little as 0.1 Gy and irreversible infertility after fractionated doses of 2.0 Gy and above [0].

In present study we have found that after irradiation with doses 0.6-1.0 Gy at the first 15 days all the investigated indicators were slightly lower from control group. But from the beginning of 30 day it becomes within normal limits. Besides, at the 65 day it was shown increasing of all parameters: daily production and total quantity of spermatozoa in testicles, total number a number of viable spermatozoa in epididymis. These results are confirmed also by other researches [0] that together with negative effect of IR on germ cells and reproductive function of animals, in the range of doses 0.1-0.8 Gy, ap-

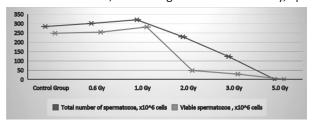


Figure 1 - Comparison between total number of spermatozoa and viable quantity in epididymis on 65 th days of experiment.

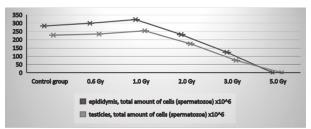


Figure 2 - Comparison of sensibility of epididymis and testicles of total quantity of spermatozoa x106 (cells) on the 65th day after irradiation.

pears radio stimulation effect, that intensifies fertilizing ability of sperm. This effect can be carried out by mobilization of stem spermatogonia and their accelerated proliferation. But at further observation even after low-dose irradiation of males at the offspring were detected decreased ability to fertilizing, congenital anomalies, induction of cancerogenesis [0].

The impact of ionizing radiation on spermatogenesis was widely investigated in laboratories and there is certain evidence concerning studies conducted on people. In 1970 American scientists carried out the study on prisoners given their voluntary consent to examine the effect of roentgen rays on testicles and spermatogenesis in particular. In this research was shown, that the dose of 0,11 Gy caused reduction of number of spermatozoa. At that time, 3.0-5.0 Gy resulted in irreversible sterility [0]. These results were confirmed in our study on rats which revealed that after single total irradiation with dose 2.0-5.0 Gy, impact of IR is damaging during whole experiment and indicators are significantly decreased.

Abuelhija et al. concluded in their research, that exist abundant dissimilarities between rat strains in spermatogenesis recuperation after exposure to 5.0 Gy radiation at 10 weeks of experiment. Testicular reaction of studied animals varies depending on breeds and species. Brown-Norway rats are easily harmed to the gonadotoxic impact of radiation and there was not detected recuperation of spermatogenesis. Advancement

of spermatogenesis recuperation of spontaneously hypertensive rats that is comparable to the prolonged delays in recovery of human spermatogenesis after impact of cytotoxic factor [0].

Long-term exposure to high levels of IR may produce testicular atrophy thereby shutting of spermatogenesis and lower doses can cause just pathology of motility of spermatozoa [0].

Conclusions

- 1. Sensitivity of testicles and epididymis to irradiation in rats is dose dependent and manifests equally in both sperm production organs.
- 2. After irradiation with doses of 0.6-1.0 Gy on the 30th and 65th day of experiment was observed increasing of studied indicators that can be explained as hormesis effect.
- 3. Total single irradiation of rats with doses of 2.0-5.0 Gy causes decreasing of studied indicators.

The prospect of further scientific research. Although, carried out studies have disclosed voluminously abnormalities of sperm production of rats after irradiation, further studies and scrupulous investigation of male population are required in upcoming years with the aim to prohibit the development of demographic crisis and risk of fertilization of egg by defective father's genetic material.

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ВПЛИВ ГОСТРОГО ТОТАЛЬНОГО ГАММА-ОПРОМІНЕННЯ НА СПЕРМОУТВОРЕННЯ ЛАБОРАТОРНИХ ЩУРІВ Николайчук Р. П., Клепко А. В.

Резюме. Чоловічі гонади є одними з найбільш чутливих органів до впливу різних ксенобіотиків на них, а зокрема, до іонізуючого випромінювання. На сьогодні можливість впливу останнього значно зменшилась завдяки розробці ряду заходів, спрямованих на запобігання та захист у промисловості, а також набутих навичок роботи з джерелами радіації. Однак, збільшення виробництва спеціальних матеріалів, що розщеплюються,

випробування різних атомних пристроїв, ядерних аварій призводять до глобального забруднення довкілля радіоактивними речовинами, дії яких піддаються все населення світу. Після двох, найбільших у світі, ядерних катастроф у Чорнобилі (1986) та Фукусімі (2011) за останні кілька років багато дослідників почали вивчати вплив низькодозового опромінення на параметри спермограми. На жаль, до цього часу дані суперечать і потребують глибокого вивчення цієї проблеми.

Мета цього дослідження— вивчити та проаналізувати вплив радіації (залежно від дози) на параметри спермопродукції щурів після тотального одноразового опромінення.

Експерименти були проведені на статевозрілих білих лабораторних щурах чоловічої статі віком у 2,5 місяці. Вивчали вплив тотального одноразового опромінення тварин, котре було проведено на установці «РОКУС» (джерело гамма-квантів — ⁶⁰Со; потужність поглинутої дози 106,6 сГр/хв) в дозах 0,6; 1,0; 2,0; 3,0 та 5,0 Гр. Тварин декапітували через 5, 15, 30 та 65 діб після проведеного опромінення. У контрольної групи щурів із імітацією опромінення, для уникнення будь-якого попадання гамма-променів на них, використовували спеціальний захисний екран із свинцю. В певні пострадіаційні терміни по 6 тварин з кожної групи разом з 6 контрольними щурами, зважували, а потім декапітували за допомогою гільйотини під легким наркозом. З відпрепарованих тварин отримували сім'яники та епідидиміси і використовували в подальших експериментах. Після чого вивчали параметри спермопродукції опромінених тварин на різних термінах після опромінення.

Встановлено, що чутливість яєчок та епідидимісу до опромінення є дозозалежною. Спостерігали, що після опромінення дозами 0,6-1,0 Гр у тварин, що були забиті на 30-й день експерименту, відбувалось підвищення досліджуваних показників. Дане явище можна пояснити як ефект гормезису. Тотальне разове опромінення всього тіла щурів дозами 2,0-5,0 Гр спричинило значне зниження як кількісних, так і якісних показників спермопродукції.

Ключові слова: тотальне опромінення, епідидиміс, яєчка, сперматогенез, сперматозоїди.

ВЛИЯНИЕ ОСТРОГО ТОТАЛЬНОГО ГАММА-ОБЛУЧЕНИЯ НА СПЕРМООБРАЗОВАНИЕ ЛАБОРАТОРНЫХ КРЫС Николайчук Р. П., Клепко А. В.

Резюме. Мужские гонады являются одними из наиболее чувствительных органов к воздействию различных ксенобиотиков на них, а в частности, к ионизирующему излучению. После двух, самых крупных в мире, ядерных катастроф в Чернобыле (1986) и Фукусиме (2011) за последние несколько лет многие исследователи начали изучать влияние низкодозового облучения на параметры спермограммы. К сожалению, до сих пор, данные в литературе противоречат и требуют глубокого изучения этой проблемы.

Цель этого исследования – изучить и проанализировать влияние радиации (в зависимости от дозы) на параметры спермопродукции крыс после тотального однократного облучения.

Эксперименты были проведены на половозрелых белых лабораторных крысах мужского пола. Тотальное однократное облучение животных было проведено на установке «РОКУС» (источник гамма-квантов — 60 Со мощность поглощенной дозы 106,6 сГр/мин) в дозах 0,6; 1,0; 2,0; 3,0 и 5,0 Гр. Животных декапитировали через 5, 15, 30 и 65 суток после проведенного облучения. После чего изучали параметры спермопродукции облученных животных.

Установлено, что чувствительность яичек и эпидидимиса к облучению является дозозависимым. Наблюдали, что после облучения дозами 0,6-1,0 Гр у животных, которые были забиты на 30-й день эксперимента, происходило повышение исследуемых показателей. Данное явление можно объяснить, как эффект гормезиса. Тотальное разовое облучение всего тела крыс дозами 2,0-5,0 Гр вызвало значительное снижение как количественных, так и качественных показателей спермопродукции.

Ключевые слова: тотальное облучение, эпидидимис, тестикулы, сперматогенез, сперматозоиды.

IMPACT OF ACUTE TOTAL-BODY GAMMA-IRRADIATION ON SPERM PRODUCTION OF LABORATORY RATS Nykolaichuk R. P., Klepko A. V.

Abstract. Male gonads are among the most sensitive organs to different xenobiotics, especially to ionizing radiation. After two, the biggest in the world, nuclear disasters in Chornobyl (1986) and Fukushima (2011) for the last few years many researches began to study effect of low-dose IR on sperm parameters. Unfortunately, till this time, data are controversy and need deep study of this problem.

Aim of this study was to study and to analyse impact of total single exposure to radiation (dose dependent) on parameters of sperm production of rats.

Studying of total X-irradiation on laboratory rats of male sex has been performed. Rats were X-irradiated by five doses 0.6; 1.0; 2.0; 3.0 and 5.0 Gy, respectively, with dose rate 106,6 cGy/min. The animals were decapitated 5, 15, 30 and 65 days after total irradiation and sperm production parameters were analyzed.

It has been established that sensitivity of testicles and epididymis to irradiation in rats is dose dependent. After irradiation with doses of 0.6-1.0 Gy from the 30 days of experiment was observed increasing of studied indicators that can be explained as hormesis effect. Total single irradiation of rats with doses of 2.0-5.0 Gy caused decreasing of sperm production and a significant decrease of viable spermatozoa.

Key words: total irradiation, epididymis, testicles, spermatogenesis, spermatozoa.

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